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=> s identimer 2 IDENTIMER => s tn-vnx 1 TN-VNX => d all L2ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN ΑN 2002-17928 BIOTECHDS Identifying and characterizing gene expression in samples, for TΙ identifying mRNAs expressed at different levels, comprises employing an identimer having a oligo-dT primer of a specific sequence and a detectable marker at its 5' end; gene expression identification an characterization, database and computer bioinformatic software ΑU KANE M D; DOMBKOWSKI A A; NAGEL A C GENOMIC SOLUTIONS INC PΑ PΙ WO 2002036828 10 May 2002 WO 2000-US45401 1 Nov 2000 AΙ PRAI US 2000-244933 1 Nov 2000 DTPatent LA English WPI: 2002-508123 [54] OS DERWENT ABSTRACT: AB NOVELTY - Systems for identification and characterization of gene expression in one or more samples, comprise an identimer having a

specific oligo-dT primer sequence, where the identimer comprises a

detectable marker at its 5' end.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a system (M1) for identification and characterization of gene expression in one or more samples, comprising: (a) providing one or more samples comprising one or more mRNA molecules; (b) providing an identimer comprising an oligo-dT primer of sequence, from 5' to 3' end, of (I) or (II), where the identimer also comprises a detectable marker at its 5' end; (c) contacting the mRNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the mRNA and the VNx portion of the identimer hybridizes with portions of the mRNA immediately upstream of the polyA tail; (d) reverse transcribing the mRNA to produce a first strand cDNA that includes the identimer; (e) synthesizing a second DNA strand complementary to the first strand cDNA to form a duplex; (f) cleaving the duplex with a sequence-specific cleaving agent to provide one or more duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA polymerase promoter site to one or more of the cleavage fragments; and (h) amplifying one or more ligated cleavage fragments using the identimer to produce one or more amplified fragments comprising sequences complementary to a 3' end of the mRNA; (2) a system for identification and characterization of gene expression in one or more samples, by: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); and (d) employing steps (c)-(h) of M1; (3) a system for identification and characterization of gene expression in one or more samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) -(c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed

RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more identimers comprising an oligo-dT primer of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-VNx (I) Tn-VNNN (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

BIOTECHNOLOGY - Preferred Method: The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database contacting sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

USE - The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

ADVANTAGE - The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and(d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence in formation.

 ${\tt EXAMPLE}$ - Experimental protocols are described but no results were given. (45 pages)

- CC GENETIC TECHNIQUES and APPLICATIONS, Gene Expression Techniques and Analysis; BIOINFORMATICS and ANALYSIS, Software; BIOINFORMATICS and ANALYSIS, Databases
- CT GENE EXPRESSION IDENTIFICATION, CHARACTERIZATION, IDENTIMER, OLIGO-DT DNA PRIMER SEQUENCE, DETECTABLE MARKER, RNA SAMPLE, RNA-POLYMERASE PROMOTER, CLEAVAGE FRAGMENT ASSOCIATED GENE IDENTIFICATION, DATABASE, COMPUTER BIOINFORMATIC SOFTWARE BIOINFORMATICS (21, 49)

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